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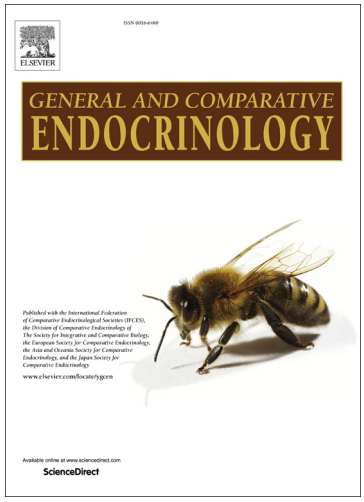

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Effects of body region and time on hair cortisol concentrations in chimpanzees (*Pan troglodytes*)

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Abstract:

Hair cortisol concentrations (HCC) are increasingly recognized as an integrated measure of the systemic cortisol secretion. Yet, we still know very little about confounding effects on HCC in animals. The present study therefore used hair from semi-wild and zoo living chimpanzees to investigate (1) intra-individual variability of HCC (body-region effect), and (2) the stability of HCC along the hair shaft (traditionally called the washout effect). Our results indicate that absolute HCC varied substantially between certain body regions, but a factor analysis revealed that these HCC differences were mainly attributable to one common source of variance. Thus, hair from all body regions provides similar biological signals and can be mixed, albeit at the cost of a lower signal-to-noise ratio. With regard to potential underlying mechanisms, we studied skin blood flow, as observed through thermal images from one chimpanzee. We found the general HCC pattern was reflected in differences in surface body temperature observed in this individual in three out of four body regions. In a separate set of samples, we found first evidence to suggest that the systematic cortisol decrease along the hair shaft, as observed in humans, is also present in chimpanzee hair. The effect was more pronounced in semi-wild than in zoo chimpanzees presumably due to more exposure to ambient weather conditions.

Keywords

Hair cortisol, stress, washout, body region

1 Introduction

Cortisol concentration measured in hair is increasingly recognized as a measure of long-term stress. Recently, the repetitive ACTH application in cows, chipmunks and lynx resulted in significantly increased HCC has provided direct evidence that hair cortisol concentrations (HCC) reflect the integrated activity of the hypothalamic-pituitary-adrenal axis (del Rosario Gonzalez-de-la-Vara et al., 2011; Mastromonaco et al., 2014; Terwissen et al., 2013). Hair cortisol analysis is increasingly applied in various circumstances, including clinical diagnosis in livestock (Comin et al., 2013), behavioral science (Dettmer et al., 2014), captive management (e.g., Carlitz et al., 2014), and conservation (e.g., Bryan et al., 2013). Hair cortisol analysis has also been applied in a growing number of mammalian species, including humans (review: Staufenbiel et al., 2013), non-human primates (e.g., Carlitz et al., 2014; Davenport et al., 2008, 2006; Dettmer et al., 2014; Fourie and Bernstein, 2011; Yamanashi et al., 2013), bears (e.g., Bechshøft et al., 2012, 2011; Macbeth et al., 2012, 2010; Malcolm et al., 2013), cows (Moya et al., 2013), horses (Anielski, 2008), dogs (Accorsi et al., 2008; Bennett and Hayssen, 2010), rock hyraxes (Koren et al., 2008), lynx (Terwissen et al., 2013) and chipmunks (Martin and Réale, 2008; Mastromonaco et al., 2014).

Despite the increased application of HCC analysis, the indicator value of HCC would be greatly improved if we knew which other factors than long-term stress affect the incorporation of and retention of cortisol in hair. Unfortunately, we insufficiently understand the factors that may confound HCC values. Various factors have been suggested, such as a systematic decline along the hair shaft (the so-called washout effect), systematic variation among body regions (body-region effect), or external contamination through sweat.

The present study attempts to fill those methodological gaps of HCC assessment in chimpanzees. While the influence of sweat is debated for humans (Grass et al., 2015; Russell et al., 2013) and horses (Anielski, 2008), it is rather unlikely to affect great ape species since their active sweat glands are mainly restricted to their palms, forehead and auxiliary organs (Montagna, 1972). We therefore examined in chimpanzees whether HCC show systematic variation across different body regions and whether HCC decreases along the hair shaft.

The washout effect has been observed in several human studies (Dettenborn et al., 2010; Gao et al., 2010; Kirschbaum et al., 2009; Skoluda et al., 2012; Steudte et al., 2011, but see Manenschijn et al., 2011; Thomson et al., 2010). It was initially suggested that structural damage of distal hair segments might permit liquids like water and cosmetics (Kirschbaum et al., 2009), or alcohol used to clean the samples before analysis (Manenschijn et al., 2011), to penetrate and wash out cortisol more easily. This would be reflected in decreasing HCC from skin-near to distal parts of the hair. While evidence for this assumption from *in vivo* studies is still pending, *in vitro* experiments by Li and colleagues (2012) showed that hair treated with shampoo solution, warm or hot water only, as well as UV-irradiation resulted in significant HCC loss, suggesting that cortisol may not only leach from the hair shaft but that it may also be degraded through UV-irradiation as was shown earlier for cannabinoids in hair (Skopp et al., 2000). Therefore, the term “waning effect” may be more appropriate than the traditionally used term “washout effect”, which was meant to indicate the leaching of cortisol by water (Kirschbaum et al., 2009).

Concerning the waning effect in animal hair, an HCC decrease by water was shown only in one *in vitro* experiment where human-like extensive wash-dry procedures resulted in decreased HCC in hair from rhesus macaques (Hamel et al., 2011). All *in*

vivo animal studies showed no HCC difference between the proximal and distal end of hair (Bennett and Hayssen, 2010; Carlitz et al., 2014; Davenport et al., 2006; Yamanashi et al., 2013). The latter findings may be in line with the *in vitro* experiments on hair of humans and rhesus macaques because the captive subjects were not exposed to rain and presumably not to strong UV-irradiation either. A systematic HCC decrease along the hair shaft should only be expected if hair is frequently exposed to water (Hamel et al., 2011), or UV-light (Li et al., 2012). However, Macbeth et al. (2010) also found no systematic decline along the hair shafts of water and light exposed free ranging grizzly bears, which raises the question whether some animal hair is inert to systematic HCC decrease along the hair shaft. The present study therefore investigated whether systematic cortisol decrease existed in chimpanzee hair and, if present, whether the effect could be explained by exposure to water only.

Concerning the body-region effect, an increasing number of studies suggest that HCC differs among various body regions in animals (Macbeth et al., 2010; Moya et al., 2013; Terwissen et al., 2013; Yamanashi et al., 2013, but see Carlitz et al., 2014; Comin et al., 2012; Macbeth et al., 2012), or between head regions in humans (Li et al., 2012). However, to our knowledge it has never been investigated whether HCC from all body regions provide similar biological information and what underlying mechanism may lead to this body-region effect. Yet, a better causal understanding of the effect may improve our understanding of the cortisol incorporation mechanisms into hair, which is still poorly understood (review: Meyer and Novak, 2012; Russell et al., 2012; Stalder and Kirschbaum, 2012).

Following the multiple compartment model of mechanisms of drug incorporation into hair (Henderson, 1993), cortisol in hair is discussed of being enriched from sweat

cortisol (Russell et al., 2013, but see Grass et al., 2015), from sebum, or from local cortisol production in the skin (Pang et al., 2014; Rousseau et al., 2007; Slominski et al., 2013, 2007) and hair follicles (Ito et al., 2005). Another mechanism suggested to contribute to the incorporation of cortisol into hair is the passive diffusion from the supplying blood capillaries into the growing hair cells (reviewed in: Meyer and Novak, 2012; Russell et al., 2012; Stalder and Kirschbaum, 2012), which would be in line with the fact that multiple weekly ACTH injections resulted in increased HCC in the hair segments representing the time of application (del Rosario Gonzalez-de-la-Vara et al., 2011; Mastromonaco et al., 2014; Terwissen et al., 2013). Following this idea, a higher activity of the hypothalamic-pituitary-adrenal axis leads to higher cortisol concentrations in the blood stream, which results in higher cortisol incorporation into the hair shaft due to increased diffusion pressure. In parallel, it is conceivable that differences in skin blood flow (SkBF) can influence the cortisol uptake into hair in a similar process. Increased SkBF may increase cortisol availability per time unit, which could result in higher diffusion rates and thus higher cortisol incorporation into the growing hair. Several studies on humans and various animal species have documented differences in SkBF between body regions using laser Doppler velocimetry or photopulse plethysmography (Monteiro-Riviere et al., 1990; Tsuchida, 1987; Tur et al., 1983). Because higher SkBF leads to increased heat dissipation, differences in SkBF can be indirectly measured as skin temperature (Rubinstein and Sessler, 1990). In order to test this skin blood flow hypothesis, the present study compared the HCC in four different body regions with their skin temperatures in one female chimpanzee.

We obtained hair cortisol measures from European zoo chimpanzees as well as from semi-wild living sanctuary chimpanzees (Ngamba Island, Uganda) from various body

regions in two consecutive years. Longitudinal segmentation of hair samples allowed investigating HCC measures along the hair shaft. Because the semi-wild chimpanzees were far more exposed to sun and rain than the zoo-living conspecifics, we could also examine whether this difference affected the waning effect.

2 Methods

2.1 Hair sampling and animals

Hair samples were cut from semi-wild living chimpanzees from the Ngamba Island (NI) sanctuary (Uganda) during anesthesia for the annual routine health checks in late February to early March 2011 (18 males aged 8-28 years, 20 females aged 5 to 28 years) and in late March 2012 (1 male aged 20, 9 females aged 9 to 25 years). These animals live in a 40 ha forest during the day and are encouraged with food to sleep in the roofed holding facilities at night. Hair samples were cut from six different body regions per individual (right and left dorsal forearm, center of back, right and left shoulder blade, middle of chest; there were 9 missing samples: right or left shoulder blade in 5 animals, right forearm in 1 animal, back in 1 animal, back and chest in 1 animal). For investigations on HCC stability along the hair shaft, additional hair samples were collected from 78 chimpanzees from 19 European zoos. However, only 24 samples from 13 zoos provided sufficient length and amount of material to be included in the segmentation study. Those samples were cut from the shoulder region (3 males aged 16 and 33 years, 6 females aged 3 to 43 years), back (3 males aged 30 and 41 years, 3 females aged 14 to 17 years), forearm (5 males aged 10 and 43 years) or combed from across several regions (3 males aged 17 and 36 years, 1 females aged 17 years) either in cooperation with well-trained animals (n = 16) or during anesthesia (n = 5) due to medical treatment.

2.2 Hair cortisol analysis

For studies on body-region effect, the 3 cm of hair proximal to the skin were analyzed, whereas for investigations on HCC stability along the hair shaft, the 4 cm of hair proximal to the skin were cut into 1-cm-segments. Here, all hair strands that provided sufficient length and amount of material were included in the analysis ($n_{Zoo} = 24$, $n_{NI2011} = 46$, $n_{NI2012} = 25$).

For cortisol analysis, all subsamples were washed twice for three minutes with 3 ml isopropanol. The air-dried hair was then minced into 3-5 mm pieces and 6 mg of this 10-50 mg pool were incubated with 1.8 ml methanol for 17 h in a glass tube. 1.6 ml of the extract were transferred into another glass tube, dried and re-suspended in 150 µl Aqua bidest. Cortisol concentrations were determined using a commercially available immunoassay with luminescence detection (LIA, IBL-Hamburg, Germany). Intra- and inter-assay coefficients of variation of this assay are below 8%.

2.3 Heat dissipation measures

Thermal images from the front and the back were taken from one female zoo chimpanzee using a thermographic camera (Vario Cam® high resolution, InfraTec GmbH, Dresden, Germany). The Software IRBIS ® 3 (InfraTec) was employed to extract temperature measures from the chest, forearm, shoulder blade and back according to the hair sampling points from the Ngamba Island chimpanzees. Measure points were directed to areas with little hair, which exhibited the highest temperature in order to avoid false measures due to the insulating effect of fur. For each body region, the mean temperature with standard deviation was derived from a small circular area (supplemental material, Figure s1).

For this study, it was not possible to add more thermal images from other animals because either chimpanzees had too much fur which prevented us from obtaining

clear measures or there was no thermographic camera with high resolution available in the facilities.

2.4 Statistical analysis

Hair cortisol data was not found to be normally distributed. Fourth root transformation reduced the skewness of distribution most effectively and was applied prior to data analyses (Miller and Plessow, 2013). For descriptive purposes, information on mean values and standard deviations are presented in original units (pg/mg). All analyses were performed using R 3.1.1 (R Core Team, 2015) statistical software.

2.4.1 HCC stability along hair shaft

Mixed-effects linear regression models were employed to assess potential HCC changes across the four consecutive segments in both zoo and NI samples, while accounting for systematic variance due to different chimpanzees and body regions. Thus, the stability of HCC across the four consecutive segments could be quantified by means of intra-class correlation coefficients (ICC; see Hruschka et al., 2005). The upper bound of such ICCs (i.e. ICC = 1) indicates perfect stability of HCC across segments (i.e., the rank order of HCCs from the first segments is also preserved across the subsequent segments), whereas the lower bound (ICC = 0) indicates no association between the different segments. The employed model is expressed in equation 1:

$$HCC^{0.25}_{ijk} = \beta_0 + \beta_1 \times \text{Segment} + \xi_i + \zeta_{ij} + \varepsilon_{ijk} \quad (1)$$

where β_0 denotes $HCC^{0.25}$ in the first segment, β_1 denotes the change of $HCC^{0.25}$ per subsequent segment. The random effects ξ_i and ζ_{ij} account for the variation in $HCC^{0.25}$ due to differences between individuals *i* and body-regions *j*, respectively.

2.4.2 HCC in different body regions

For descriptive purposes, Pearson correlation coefficients were calculated to investigate the HCC relationships between the different body regions. Thereafter, a confirmatory factor analysis was performed (Rosseel, 2012) to investigate whether the covariance structure of HCC across different body regions could be sufficiently accounted for by one common factor. Besides the robust likelihood ratio statistic (Bentler and Yuan, 1999), model fit was evaluated using the comparative fit index (CFI), root mean square error of approximation (RMSEA), standardized root mean square residual (SRMR) and the Bayesian information criterion (BIC). HCC differences between the investigated body regions were assessed using paired t-Tests.

3 Results

3.1 HCC stability along the hair shaft

Pair-wise comparison revealed that all four segments were highly correlated in both samples from the zoos and from Ngamba Island ($0.9 \geq r \geq 0.7$, all p's < 0.001). Mixed-effects regressions of HCC on the four consecutive 1-cm-segments revealed profound differences between change of HCC in chimpanzee hair from European zoos and those from the NI sanctuary. In zoo chimpanzees, we found a decrease of HCC across all segments that reached statistical significance ($\chi^2(1) = 4.22$, $p = 0.040$) but was numerically negligible (mean ± SD decrease of HCC per segment: 5.7% ± 18.8%; 61.1% explained variance). The intra-individual stability of HCC across the four segments amounted to ICC = 0.81. Hair samples from NI-chimpanzees showed a more pronounced cortisol decline along the hair strand ($\chi^2(1) = 72.40$, $p < 0.001$; mean ± SD decrease of HCC per segment: 20.1% ± 24.9%; 69.6% explained variance). However, the intra-individual stability of HCC across the

four segments was similar to that of the zoo samples, and amounted to ICC = 0.83. After controlling for body-region-related differences in HCC, the intra-individual stability estimate of HCC drops to ICC = 0.32. This is the proportion of HCC that can actually be attributed to environmental and individual stability across the time interval covered by the hair stands from different body regions of a single chimpanzee. The mean trajectories of the HCC decrease across segments, as traditionally reported in previous studies (Carlitz et al., 2014; Kirschbaum et al., 2009; Manenschijn et al., 2011), are visualized in Figure 1 whereas a more detailed visualization of the HCC development along segments is presented in Figure 2.

[Please insert Figure 1 here]

[Please insert Figure 2 here]

3.2 HCC in different body regions

Descriptive statistics for HCC in the different body regions are provided in Table 1. Pearson correlations were highly significant between all pairs of body regions (Table) and showed a strong effect size (Cohen, 1988). Results from the factor-analysis revealed that the HCC covariance structure could be sufficiently accounted for by only one factor ($\chi^2(2) = 2.07$, $p = 0.35$; CFI = 1.00, $RMSEA_{90\%} = 0.00 – 0.31$, SRMR = 0.02, BIC = -119.74), suggesting that HCC at different body regions were driven by one systemic factor across the time interval covered by the length of hair strand. Factor loadings indicated, that chest HCC ($\lambda = 0.90$, $p < 0.01$) contributed most substantially to this “cortisol exposure” factor, followed by shoulder and forearm HCC (both λ’s = 0.87, p’s < 0.01), and finally back HCC ($\lambda = 0.78$, $p < 0.01$). Constraining all loadings to equality did not result in a significantly worse model fit ($\Delta\chi^2(3) = 6.27$, $p = 0.10$ with $\chi^2(5) = 8.95$, $p = 0.11$, with ; CFI = 0.98, $RMSEA_{90\%} = 0.00 – 0.25$; SRMR

= 0.13, BIC = -122.56). Therefore, HCC differences between animals probably are equally manifested in hair from all body regions.

[Please insert Table 1 here.]

Besides the strong correlations, the paired t-Tests revealed that the absolute HCC differed (at least marginally) significantly between all investigated regions (all *p's* ≤ 0.06; Figure 3). HCC was significantly higher in the chest region followed by shoulder, forearm and back. A very similar pattern was observed for temperature measures based on thermal images from one chimpanzee although only three out of four body regions followed the average HCC pattern in this particular chimpanzee (Figure 4, Figure s1).

[Please insert Figure 3 and Figure 4 here.]

4 Discussion

Hair cortisol analysis is increasingly applied to measure long-term stress in animals and humans, but we still lack sufficient understanding of potentially confounding effect, such as the waning effect along the hair shaft and possible differences in steroid incorporation at different body regions. In this study, we estimated these effects using chimpanzee hairs.

Concerning the waning effect, our results revealed a strong HCC decrease towards the distal end of the hair shaft in samples from Ngamba Island chimpanzees as well as a significant though very weak effect in samples from European zoo chimpanzees. While this is in line with many human studies (Dettenborn et al., 2012; Kirschbaum et al., 2009; Skoluda et al., 2012, but see Manenschijn et al., 2011; Thomson et al., 2010), the present results oppose all previous animal studies, which reported no

systematic cortisol decrease towards distal segments (Bennett and Hayssen, 2010; Carlitz et al., 2014; Davenport et al., 2006; Macbeth et al., 2010; Yamanashi et al., 2013).

It is unlikely that differences in laboratory protocols, as suggested by Manenschijn et al. (2011), led to the discrepancy between the present and former results because the same protocol was also applied in our orangutan study, in which no cortisol decrease along 15 cm long hair shafts was observed (Carlitz et al., 2014). It is also unlikely that this is a species specific result since Yamanashi et al. (2013) did not find a waning effect in chimpanzees either. There might be a difference between captive animals that are sheltered from ambient weather conditions and (semi-) wild animals that are exposed to ambient weathers. However, Macbeth et al. (2010), the only study investigating HCC along the hair shaft in wild animals, did not find a waning effect either. Yet, it is conceivable that the formerly applied statistical methods (repeated-measures ANOVA) were not sensitive enough because it pools valuable information. Interestingly, a repeated-measures ANOVA would not have brought to light the small waning effect in the present samples from zoo chimpanzees but only in the NI samples (data not shown). The detailed graph of cortisol changes along the hair shaft (Figure) illustrates that in zoo samples the effect is masked by considerable background noise and is only visible in the second and third segment if samples with greater initial HCC are included. This may be due to the fact that individual samples with low (initial) HCC are more likely to show an increase or no change over time rather than a decrease of HCC due to bottom effects. Furthermore, it is conceivable that small quantities of cortisol exhibit stronger bonds to the hair matrix and are less likely to be removed by external factors than larger quantities of cortisol. Macbeth et al. (2010) already mentioned the possibility that a waning effect

300 was not detectable in their study on wild grizzly bears because of low HCC in their
301 samples.

302 In search of underlying mechanisms for the systematic cortisol decrease observed in
303 the present study, internal factors (i.e., stress related) as well as external factors
304 (e.g., rain, sun) have to be considered. Concerning internal factors, it seems unlikely
305 that stress levels decreasing systematically across the time period covered by the
306 hair segments affected the whole NI group in two consecutive years and the
307 inhomogeneous zoo group alike, resulting in systematic cortisol decrease along the
308 hair shaft. Concerning external factors, water (hair washes in humans or rain in
309 animals) has often been suggested as the primary source of cortisol decrease along
310 the hair shaft, which is why this effect was called washout effect (Kirschbaum et al.,
311 2009). To date, however, only two *in vitro* studies (Hamel et al., 2011; Li et al., 2012),
312 revealed a direct relationship between exposure to water and HCC decrease along
313 the hair. Moreover, the one *in vivo* study (Dettenborn et al., 2012), which tried to
314 correlate the number of weekly hair washes in humans with the waning effect along
315 the hair shaft, may not have found the effect because of too many other factors that
316 add noise to the data.

317 The *in vitro* experiment from Li et al. (2012) suggests that water and UV-irradiation
318 can reduce HCC independently, although *in vivo* data for the effect of UV-irradiation
319 on HCC is still pending. The pronounced cortisol decrease in NI samples and the
320 very weak effect in samples from zoo animals could also be interpreted as the result
321 of difference in exposition to UV-irradiation (moderate European vs. strong equatorial
322 sun), difference in exposition to water (negligible in European zoos vs. routinely in NI
323 animals), or differences of a combination of both. However, this interpretation at
324 present remains speculative because of the higher noise level (i.e., fluctuation of the

environment) in the inhomogeneous zoo samples, and a weakened hair structure from one factor with cortisol removal, or degradation from another factor (*cf.* Manenschijn et al., 2011) can also not be excluded. Thus, the underlying mechanisms for the waning effect remain to be investigated as well as the question to what extent hair samples from the European zoo animals are affected.

Despite the presence of the waning effect our results showed a strong intra-sample stability in both groups and all segments provided similar biological information (although absolute HCC differed between segments). Thus, if animals are exposed to similar ambient conditions, the effect can be 'controlled for' using the same length of hair throughout the study.

Concerning the body-region effect, our results revealed that absolute HCC differed significantly between body regions, which is in line with previous studies (Macbeth et al., 2010; Moya et al., 2013; Terwissen et al., 2013; Yamanashi et al., 2013). In addition, and similar to the chimpanzee study from Yamanashi et al. (2013), our results revealed strong correlations between body regions. The present data extend previous research showing that HCC measures of all body regions were mainly driven by one common factor. Thus, HCC in all body regions appear to convey information about the same biological entity, and it is likely that this biological entity is an excellent representation of the systemic cortisol secretion as suggested by an increasing body of literature in animals (e.g., Carlitz et al., 2014; del Rosario Gonzalez-de-la-Vara et al., 2011; Malcolm et al., 2013; Mastromonaco et al., 2014; Terwissen et al., 2013) and humans (e.g., O'Brien et al., 2013; Stalder et al., 2014). Assuming no asymmetry of hair samples from different regions, controlling for body region could be omitted if a large enough sample from random body regions is

available, even though it inevitably will reduce the signal-to-noise ratio. Thus, it is possible to use shed hair samples as a mixture of various body regions.

In an attempt to explain the underlying mechanism of the effect of body region, we found that skin temperature, which is a measure of skin blood flow (Rubinstein and Sessler, 1990), increased in the same order as the mean HCC levels from the 38 NI chimpanzees (back<forearm<shoulder<chest). Even though there is an unexplained mismatch for one body region between skin temperature and HCC in the particular subject for which skin temperature was recorded, it is conceivable that higher skin blood flow indicates a greater blood supply to the hair root per unit time, which might result in a higher diffusion rate and therefore in more cortisol incorporation into the hair shaft. Indeed, HCC patterns of body regions described earlier for chimpanzees (side > back > elbow; Yamanashi et al., 2013) and for orang-utans (shoulder > stomach = back > wrist, although this pattern was not significant; Carlitz et al., 2014) also seem to follow the skin temperature gradient illustrated in the thermal images of one chimpanzee (see supplemental material Figure s1). While these results encourage further investigations of the relationship between HCC and skin blood flow, more data with temperature measures from more animals as well as from more body regions are necessary to verify this skin blood flow hypothesis. Yet, future work should also consider a potential interaction between the systemic and local cortisol production as suggested by Keckeis et al. (2012), although Pang et al. (2014) found that local cortisol production in the skin was reduced with increased systemic cortisol concentration.

In conclusion, this study presents first evidence that the waning effect, so far only reported for human hair, influences HCC along the hair shaft of chimpanzees. Nonetheless, all segments provided similar biological information. Regarding the

374 body-region effect, our results confirm that absolute HCC differ between body regions
375 and extend previous research by showing that HCC in all body regions convey similar
376 biological information, presumably the systemic cortisol secretion. In conclusion,
377 shed hair from various unknown body regions can well be used in observational
378 studies at the cost of a lower signal-to-noise ratio.

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391

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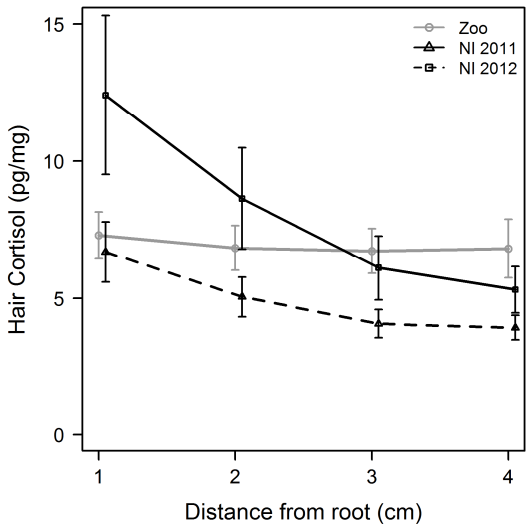

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586 Figure 1: Mean hair cortisol concentrations (± SE) of the four proximal 1-cm-segments from 24
587 European zoos-living chimpanzees (gray line, *n* = 24 samples), 38 chimpanzees from Ngamba Island
588 (Uganda) in 2011 (dashed black line, *n* = 46 samples), and from 7 Ngamba Island chimpanzees in
589 2012 (solid black line, *n* = 25 samples).

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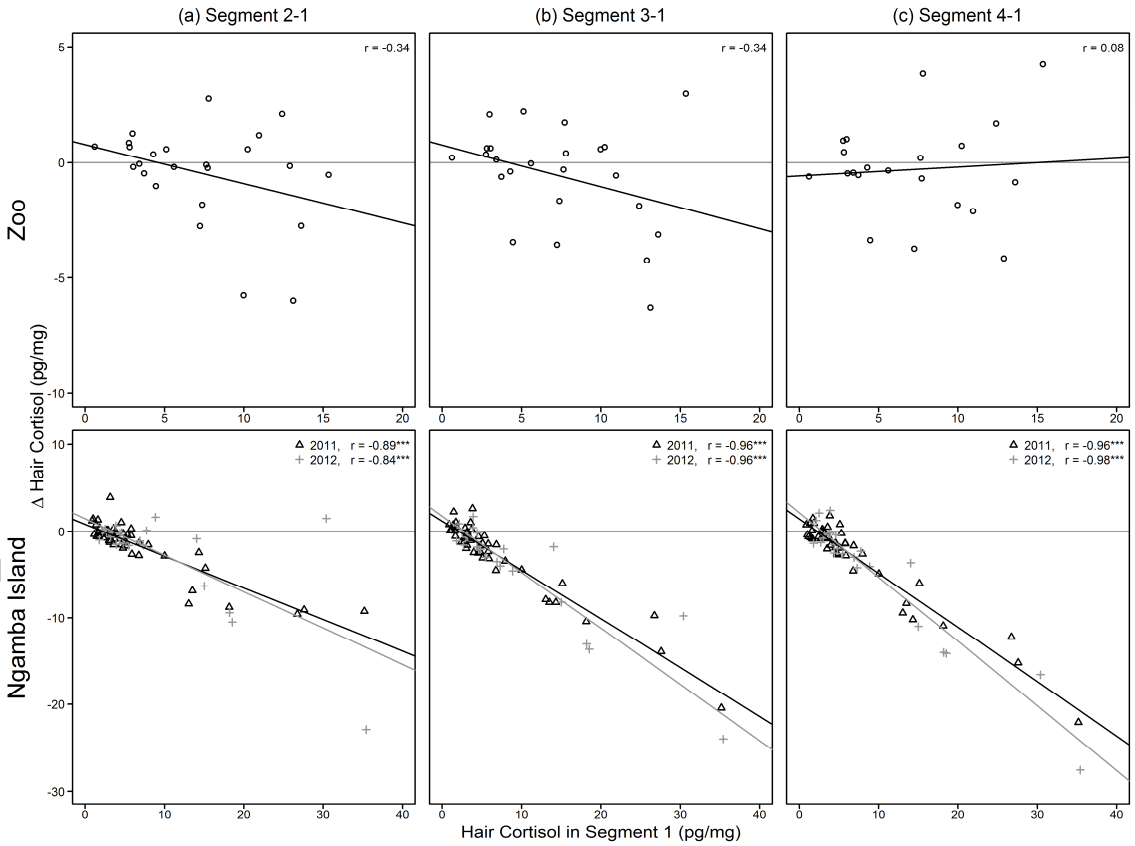

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Figure 2: Detailed illustration of the hair cortisol changes along four consecutive 1-cm-segments for samples from European zoo chimpanzees (n = 24) and from semi-wild living chimpanzees from the Ngamba Island (NI) Chimpanzee Sanctuary, Uganda (hair samples from 2011, n = 46; hair samples from 2012, n = 25). Negative y-values indicate that cortisol concentrations (HCC) in segment 2 (a), segment 3 (b) or segment 4 (c) was smaller than cortisol concentration in segment 1. Cortisol decrease towards the distal end of hair was present in both NI ($\chi^2(1) = 72.40$, $p < 0.001$) and zoo samples ($\chi^2(1) = 4.22$, $p = 0.040$), but only NI samples exhibited significant correlations between HCC in segment 1 and the loss of HCC across all following segments. ***$p < 0.001$

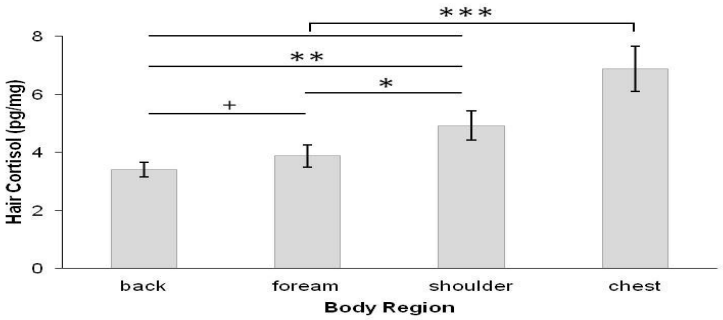

Figure 3: Bar plot illustrating the mean hair cortisol concentrations (±SE) of four body regions from 48 samples sets from semi-wild ranging chimpanzees from the Ngamba Island chimpanzee sanctuary (Uganda; 2011: n = 38, 2012: n = 10). Each value is an integrated measure of the three cm of hair closest to the skin. Forearm and shoulder show the mean concentrations of the right and left side. + p = 0.06, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

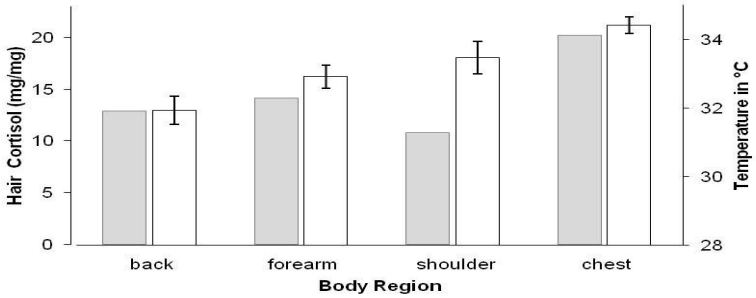

Figure 4: Bar plot illustrating the hair cortisol concentration (gray bars) and skin temperature (± SD, white bars) of four body regions from one female zoo chimpanzee. Each cortisol value is an integrated measure of the three cm of hair closest to the skin. Temperature measures were measured using thermal imaging. Forearm and shoulder samples were taken from the right side.

615 Table 1: Pearson correlation coefficients of hair cortisol concentrations of four body regions from 48
616 chimpanzees. Point estimates are listed below the main diagonal of the matrix, whereas their
617 bootstrapped 95% confidence regions are listed above the main diagonal.

	back	forearm	shoulder	chest
N	46	48	47	47
Mean	3.41	3.88	4.92	6.88
SD	1.73	2.69	3.48	5.31
Minimum	0.18	0.6	0.97	1.09
Maximum	9.28	16.82	17.01	31.49
Correlation (back)	1	[0.62, 0.82]	[0.53, 0.77]	[0.56, 0.80]
Correlation (forearm)	0.73	1	[0.57, 0.87]	[0.60, 0.88]
Correlation (shoulder)	0.67	0.75	1	[0.68, 0.89]
Correlation (chest)	0.69	0.78	0.81	1

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620 **Highlights:**

621 Cortisol concentrations decreased significantly along the hair shaft of chimpanzees.

622 All segments along the hair shaft provided similar biological information.

623 Absolute hair cortisol concentrations (HCC) differed between body regions.

624 HCC among various body regions conveyed similar biological information.

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